Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information for:

The interplay between crystallinity and the levels of Zn and carbonate in synthetic microcalcifications directs thyroid cell malignancy

Lotem Gotnayer[1], Yarden Nahmias[1], Gabriel Yazbek Grobman[1], Lonia Friedlander[2], Dina Aranovich[1], Uri Yoel[3,4], Netta Vidavsky[1,2*]

[1]Department of Chemical Engineering, Ben-Gurion University of the Negev, Beer Sheva, Israel
[2]Ilse Katz Institute for Nanoscale Science & Technology, Ben-Gurion University of the Negev, Beer Sheva, Israel
[3]Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel
[4]Endocrinology, Soroka University Medical Center, Beer Sheva, Israel
[*] Corresponding author

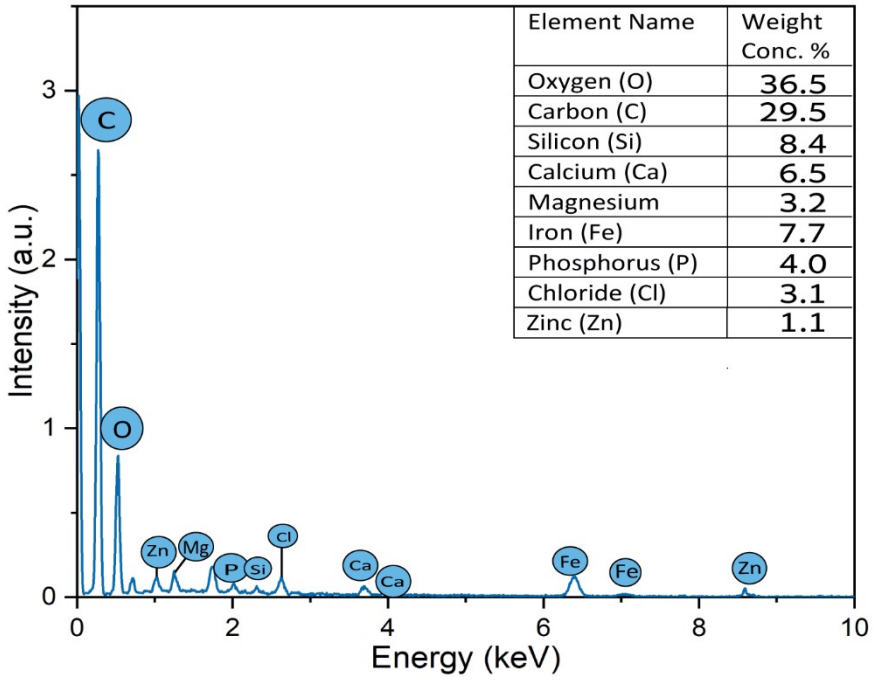


Figure S1. The elemental composition of an FNA-derived MC extracted from a cancerous thyroid nodule, as shown in Figure 1 a, was assessed using EDS analysis.

Table S1. Areas and ratios of carbonate and phosphate peaks in MC analogs determined through FTIR measurements.

Zn fraction in the MC analog (wt%)	CO_3 **area**	PO_4 **area**	CO_3/PO_4
0	0.128	10.7	0.012
0.74	0.0489	12.3	0.0040
1.2	0.0737	9.28	0.0079
2.6	0.0609	11.4	0.0054
5.2	0.0308	5.95	0.0052

The integration range was calculated between 850 and 890 cm^{-1} for the carbonate peak and between 900 and 1200 cm^{-1} for the phosphate peak.

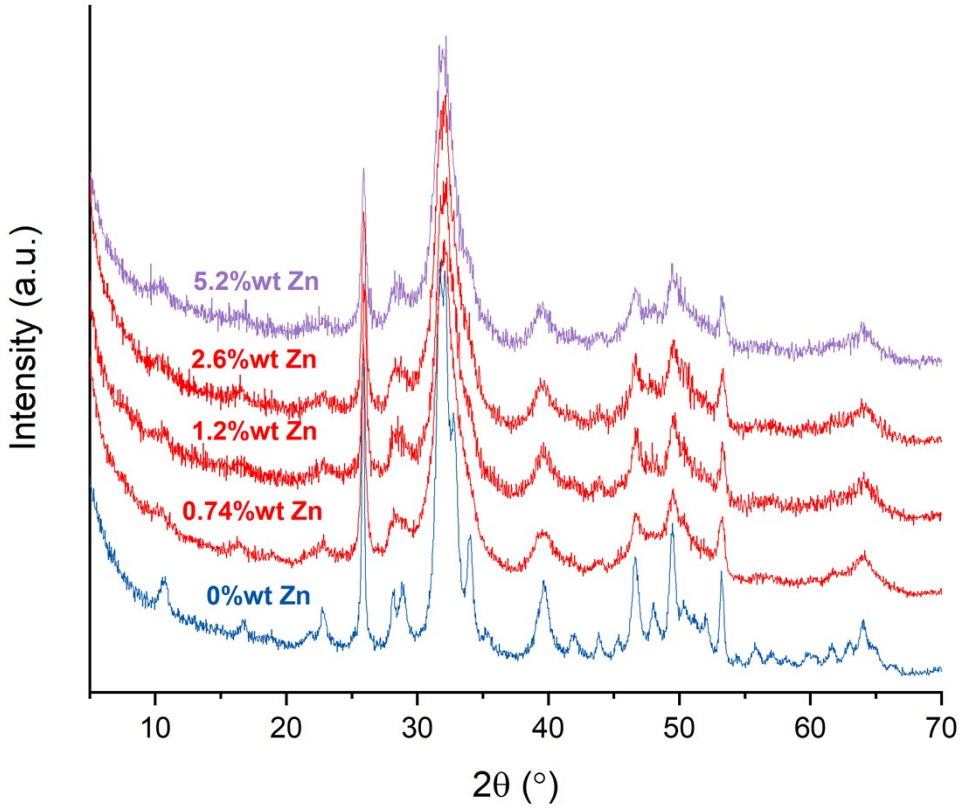


Figure S2. X-ray diffractograms of the MC analogs. Peak broadening is associated with a Zn increase.

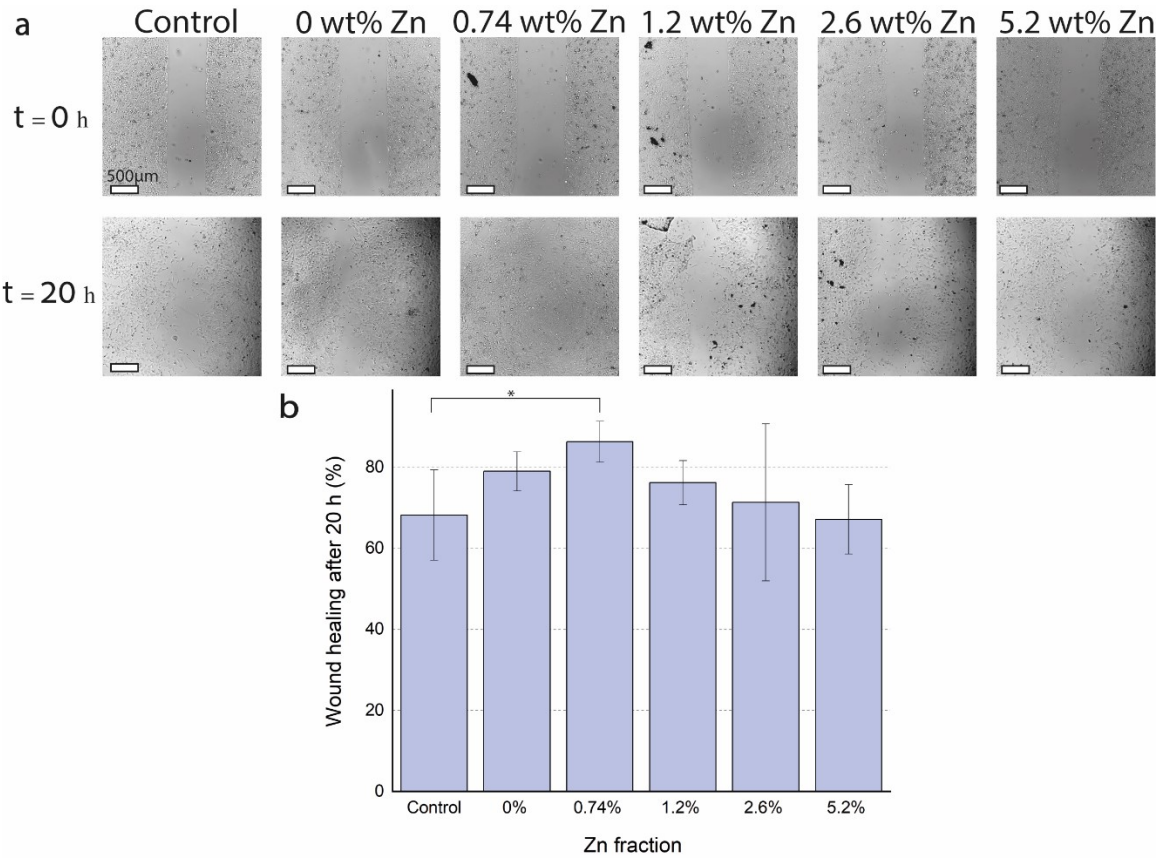
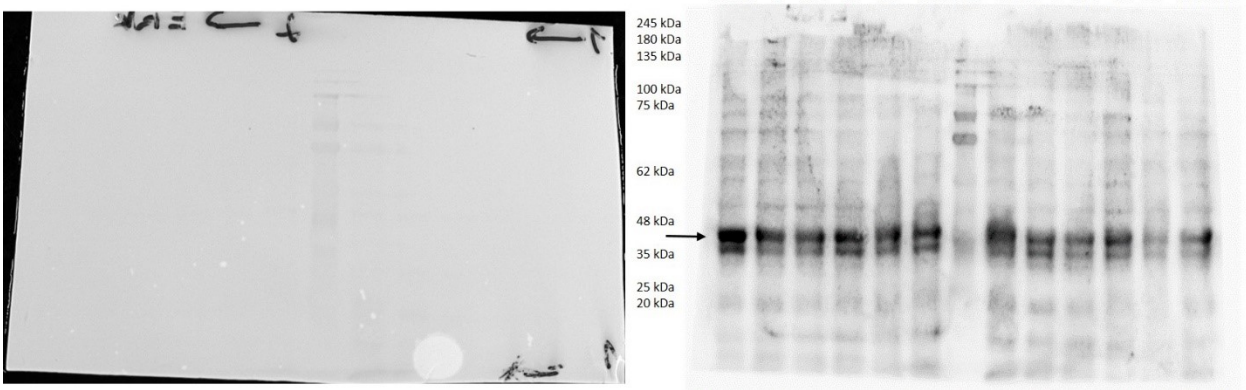
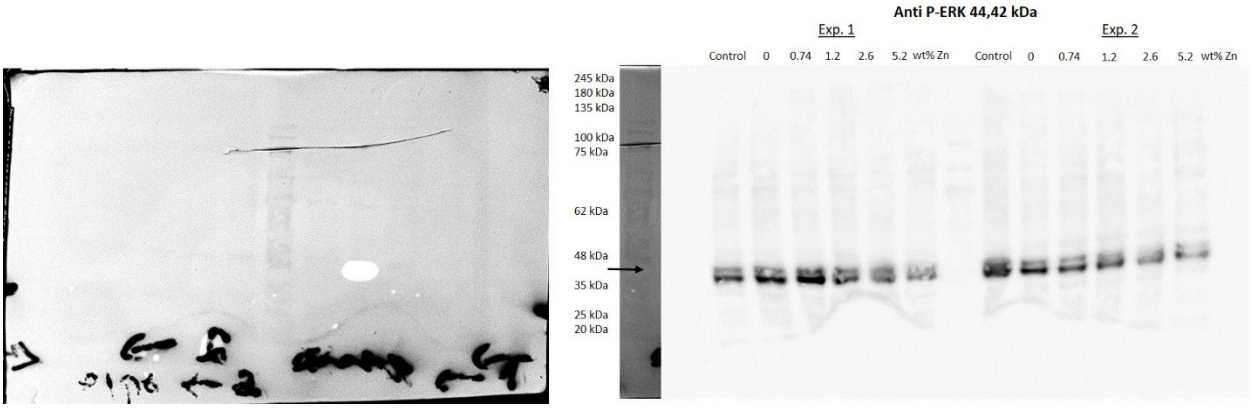
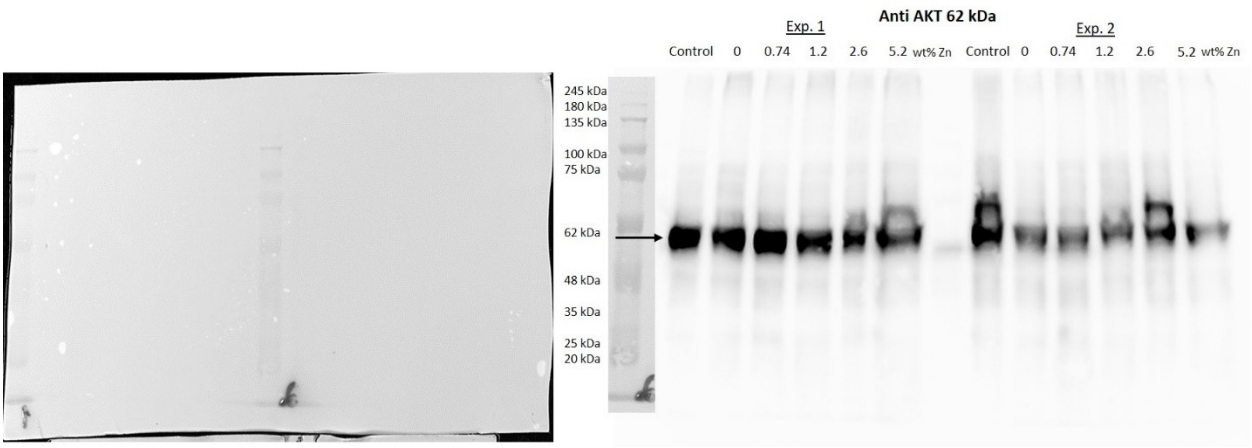


Figure S3. Cell migration assay according to Zn content in the MC analogs. **a**. Light microscopy imaging of the wound healing assay. **b**. The extent of cell migration, quantified as wound coverage, was evaluated after 20 hours of culture with MC analogs. Wound healing was calculated as the gap area covered by cells after 20 h divided by the gap area at time 0. Error bars represent the standard deviation. *P<0.05.


Anti ERK 40, 42 kDa
Exp. 1
Exp. 2
Control 0 0.74 1.2 2.6 5.2 wt% Zn
Control 0 0.74 1.2 2.6 5.2 wt% Zn
245 kDa
180 kDa
135 kDa
100 kDa
75 kDa
62 kDa
48 kDa
35 kDa
25 kDa
20 kDa


Anti P-ERK 44,42 kDa
Exp. 1
Exp. 2
Control 0 0.74 1.2 2.6 5.2 wt% Zn
Control 0 0.74 1.2 2.6 5.2 wt% Zn
245 kDa
180 kDa
135 kDa
100 kDa
75 kDa
62 kDa
48 kDa
35 kDa
25 kDa
20 kDa


Anti AKT 62 kDa
Exp. 1
Exp. 2
Control 0 0.74 1.2 2.6 5.2 wt% Zn
Control 0 0.74 1.2 2.6 5.2 wt% Zn
245 kDa
180 kDa
135 kDa
100 kDa
75 kDa
62 kDa
48 kDa
35 kDa
25 kDa
20 kDa

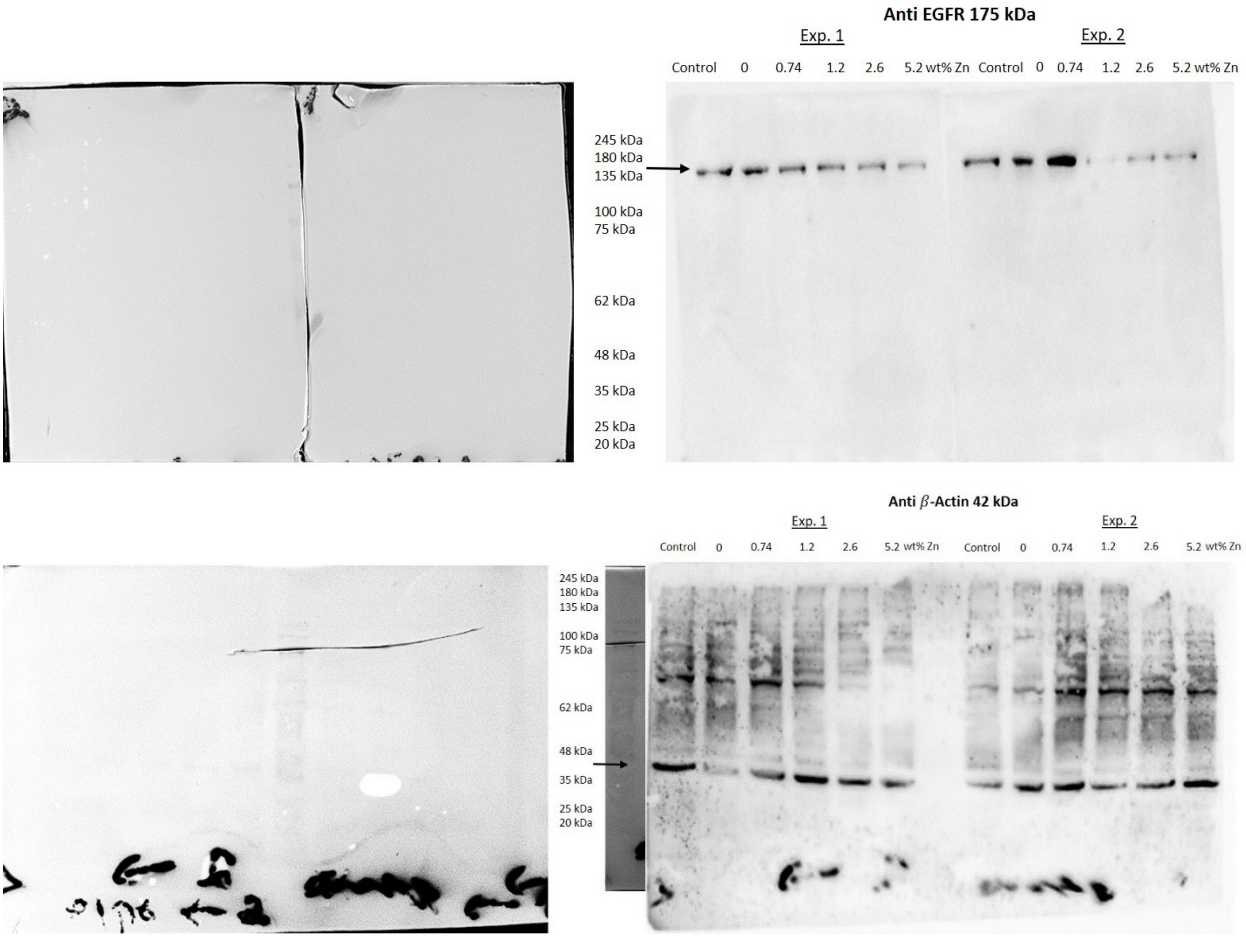


Figure S4. Western blot with anti-ERK, anti-p-ERK, anti-AKT, anti-EGFR, and anti-β-actin antibodies. Samples of two individual experiments, each containing 30 μg of total protein, were loaded onto four gels in the same order and electrophoresed simultaneously. After the transfer, four duplicates of the membranes were obtained and incubated with anti-ERK, anti-p-ERK, anti-AKT, anti-EGFR and anti-Actin diluted at 1:1000.